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Ginseng Treatment Reduces Bacterial Load and Lung Pathology in Chronic *Pseudomonas aeruginosa* Pneumonia in Rats

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The predominant pathogen in patients with cystic fibrosis (CF) is *Pseudomonas aeruginosa*, which results in a chronic lung infection associated with progressive pulmonary insufficiency. In a rat model of chronic *P. aeruginosa* pneumonia mimicking that in patients with CF, we studied whether the inflammation and antibody responses could be changed by treatment with the Chinese herbal medicine ginseng. An aqueous extract of ginseng was injected subcutaneously, and cortisone and saline were used as controls. Two weeks after challenge with *P. aeruginosa*, the ginseng-treated group showed a significantly improved bacterial clearance from the lungs ($P < 0.04$), less severe lung pathology ($P = 0.05$), lower lung abscess incidence ($P < 0.01$), and fewer mast cell numbers in the lung foci ($P < 0.005$). Furthermore, lower total immunoglobulin G (IgG) levels ($P < 0.01$) and higher IgG2a levels ($P < 0.025$) in serum against *P. aeruginosa* sonicate and a shift from an acute type to a chronic type of lung inflammation compared to those in the control and cortisone-treated groups were observed. These findings indicate that ginseng treatment of an experimental *P. aeruginosa* pneumonia in rats promotes a cellular response resembling a TH1-like response. On the basis of these results it is suggested that ginseng may have the potential to be a promising natural medicine, in conjunction with other forms of treatment, for CF patients with chronic *P. aeruginosa* lung infection.

A distinctive feature of patients with cystic fibrosis (CF) is the chronic *Pseudomonas aeruginosa* lung infection which leads to a slowly progressive damage of the lung parenchyma and eventually respiratory insufficiency (7, 27). The chronic *P. aeruginosa* lung infection is characterized by a significant antibody response and the infiltration of numerous polymorphonuclear leukocytes (PMNs) resembling a TH2-like response (15). This situation results in the inability of the host to clear the bacteria efficiently, and the elastase from PMNs plays an important role in the damage of the lung tissues of CF patients (7, 27). The pathogen is an alginate-producing mucoid strain of *P. aeruginosa*, and the infection in CF patients is rarely eradicated by antibiotics (7, 27). It is therefore important to find new and efficacious measures for the treatment of *P. aeruginosa* pneumonia in CF patients. We have previously found that immunization with *P. aeruginosa* vaccines and adjuvants or that treatment with gamma interferon or the Chinese herbal medicine *Daphne giraldii* Nitsche could decrease the inflammatory response and enhance bacterial clearance in a rat model (13, 15, 16, 29). In the present study we investigated the effects of the Chinese herbal medicine ginseng on the chronic *P. aeruginosa* pneumonia in rats and found that ginseng treatment of these animals promoted a TH1-like response and reduced the bacterial load significantly in the lungs of the treated animals.

MATERIALS AND METHODS

Animals. Sixty female Lewis rats (Charles River, Würtzburg, Germany) that were 7 weeks old and that had a body weight of approximately 150 g were used.

Challenge strain. *P. aeruginosa* PAO 579 (kindly provided by J. R. W. Govan, Department of Bacteriology, Medical School, University of Edinburgh, Edinburgh, United Kingdom), which stably maintains a mucoid phenotype and which is International Antigenic Typing System type O:2/5, was used in our study.

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Immobilization of *P. aeruginosa* in seaweed alginate beads. Immobilization of *P. aeruginosa* in seaweed alginate beads was done as described previously (14, 28). In brief, 1 ml of the *P. aeruginosa* bacterial culture was mixed with 9 ml of seaweed alginate (60% guluronic acid content), and the mixture was forced once with air through a cannula into a solution of 0.1 M $CaCl_2$ in 0.1 M Tris-HCl buffer (pH 7.0). The suspension was adjusted to yield 10^9 CFU/ml, and the yield was confirmed by colony counts.

Treatment protocol. Rats were divided into three groups, each comprising 20 animals.

(i) Group 1. *Panax ginseng* C. A. Meyer (ginseng) (11) powder was provided by Millingwang Limited, Jilin, People's Republic of China. A water extract of ginseng was prepared by the following method. A total of 2.5 g of ginseng powder was mixed with 100 ml of distilled water at room temperature for 20 min, and then the mixture was heated at 90°C for 30 min and then filtered through sterile filter paper twice before use (final concentration, 25 mg of the equivalent of dry powder per ml). The concentration of protein in the ginseng extract was measured by a Bio-Rad method, and the result was 3.5 mg/ml. The endotoxin-like activity was measured by a *Limulus* amoebocyte lysate assay (2), and the level was 60 ng/ml, which is 1,660 times lower than the dosage of lipopolysaccharide (LPS) that we used in another study (20). The ginseng solution was injected subcutaneously by using a dosage of 25 mg/kg of body weight once a day for 10 days. The dosage was decided on the basis of a ginseng dosage pilot study.

(ii) Group 2. Hydrocortisone (cortisone; Upjohn s.a., Puurs, Belgium), a commercial product with a concentration of 50 mg/ml which is commonly used as an anti-inflammatory agent, was used as a control drug in the study. Cortisone was administered subcutaneously at a dosage of 25 mg/kg of body weight once a day for 10 days. The dosage was determined on the basis of the dosage used in the treatment of CF patients with *P. aeruginosa* infection (5).

(iii) Group 3. Group 3 was the control group. Sterile saline (0.9%) was injected subcutaneously at 1 ml/kg of body weight once a day for 10 days.

In all groups the injections were started on the same day as challenge with *P. aeruginosa* alginate beads.

The anti-*P. aeruginosa* activities of the drugs were screened by an agar disk diffusion test. Blood agar plates (State Serum Institute, Copenhagen, Denmark) were seeded with *P. aeruginosa* PAO 579 to obtain semiconfluent growth, 10-mm-diameter paper disks containing 25 μl of undiluted solution of each drug were placed on the seeded plates, and the plates were incubated overnight at 37°C. Antibacterial activity was recorded as the inhibition zone around the disks after incubation.

Challenge procedures and blood samples. At the time of challenge, all rats were anesthetized subcutaneously with a 1:1 mixture of etomidate (Janssen, Birkerød, Denmark) and midazolam (Roche, Hvidovre, Denmark) at a dose of 1.5 ml/kg of body weight and were tracheotomized (13). Intratracheal challenge with 0.1 ml of *P. aeruginosa* (10^9 CFU/ml) in alginate beads was performed as described previously (13). The incision was sutured with silk, and the wounds healed without any complications. Fourteen days after challenge, all rats were

TABLE 1. Median numbers of CFU of *P. aeruginosa* in rat lungs 14 days after intratracheal challenge

Treatment group (no. of animals)	Median (range) bacterial count (CFU)/lung	No. (%) of rats with <100 CFU[a]
Control (10)	8.5×10^2 (1.3×10^1–3.6×10^6)	2 (20)
Ginseng (10)	1.5×10^1 (0–2.2×10^5)[b,c]	7 (70)[d,e]
Cortisone (10)	5.6×10^3 (0.4×10^1–6.7×10^5)	2 (20)

[a] A three-by-two chi-square analysis for <100 CFU showed a significant difference between the groups ($P < 0.05$; $\chi^2 = 7.178$).
[b] $P < 0.04$ compared to the control group.
[c] $P < 0.04$ compared to the cortisone-treated group.
[d] $P < 0.025$ compared to the control group.
[e] $P < 0.025$ compared to the cortisone-treated group.

sacrificed by using 20% pentobarbital (DAK, Copenhagen, Denmark) at 3 ml/kg of body weight, and blood samples were obtained by cardiac puncture.

Macroscopic description of the lungs. The lungs from all rats were macroscopically described in situ and after removal from the thoracic cavities. The pathological changes in the lungs were divided into four different groups according to the severity of the inflammation, as described previously (13–15): group I, normal lungs; group II, swollen lungs, hyperemia, small atelectases (3 by 3 mm); group III, pleural adhesions and atelectasis (<5 by 8 mm); and group IV, abscesses, large atelectases, and hemorrhages.

Histopathologic scoring. Half of the animals in each group were examined for lung histology, as described previously (13). The lung pathology was divided microscopically into four scoring groups according to the severity of the inflammation, as follows: 1, normal; 2, mild focal inflammation; 3, moderate to severe focal inflammation with areas of normal lung tissue; and 4, severe inflammation to necrosis or severe inflammation throughout the lung. The cellular alterations were assigned to the acute and chronic inflammation groups by a scoring system based on the proportion of PMNs and mononuclear leukocytes (MNs) in the inflammatory foci. Acute inflammation was defined as an inflammatory infiltration dominated by PMNs (PMNs ≥ 90%, MNs ≤ 10%), whereas chronic inflammation was defined as a predominance of MNs (MNs ≥ 90%, PMNs ≤ 10%), which included lymphocytes and plasma cells and the presence of granulomas (7, 13).

Toluidine blue staining was performed to detect mast cells (19) in lung tissues. Ten representative fields (magnification, ×500) were selected along the inflammatory foci to count the number of mast cells. The results were expressed as the total number of mast cells, and we classified the results into three scoring groups according to the number of mast cells: i, <150 mast cells; ii, ≥150 and ≤300 mast cells; and iii, >300 mast cells.

The histopathological evaluations were done blindly to avoid bias.

Bacteriology. The lungs of the remaining half of the animals in each group were prepared for the quantitative bacteriological examination as described previously (13). Appropriately diluted samples were plated to determine the numbers of CFU. For detection of the lower limits of the numbers of CFU, lung homogenates were prepared as follows. Three milliliters of phosphate-buffered saline was added to each set of lung tissue samples, and the mixture was then homogenized. The total volume of each lung homogenate was 4.5 ml. A total of 0.1 ml of the homogenate was plated for bacterial culture. The bacteria were thus diluted 45 times. By using a 95% confidence interval (Poisson distribution), this should yield the theoretical absolute limit of 135 bacteria per set of lung tissue samples. In order to see a minimum of 1 CFU in 95% of the lung tissue samples, the absolute number of bacteria should be >135 in a set of lung tissue samples. This means that zero means <135 bacteria in 95% of the lung tissue samples.

ELISA. Quantitation of anti-*P. aeruginosa* sonicate (PAO 579, O:2/5) antibodies of the immunoglobulin M (IgM), IgG, and IgA classes and the IgG1 and IgG2a subclasses as well as anti-*P. aeruginosa* alginate (strains 6680 and 8839) antibodies of the IgM, IgG, and IgA classes was carried out by enzyme-linked immunosorbent assays (ELISAs) as reported previously (14, 17). The antibody concentrations expressed as ELISA units were obtained by dividing the mean optical density of the samples by the mean optical density of an internal standard expressing absorbance units of between 0.30 and 0.40.

Statistical analysis. Unpaired differences in continuous data were analyzed by the Mann-Whitney U test, and categorical data were compared by the chi-square test.

RESULTS

Disk diffusion examination on blood agar plates. None of the three agents studied was found to have anti-*P. aeruginosa* activity.

Mortality. Only one rat (in the cortisone-treated group) died during the study; no deaths were found among rats in the two other groups.

Bacteriology. *P. aeruginosa* could be cultured from the lungs of most surviving rats 2 weeks after challenge (Table 1). However, the bacterial count in the ginseng-treated group was significantly lower than those in the two control groups ($P < 0.04$), and 7 of 10 rats in the ginseng-treated group were found to be infected with fewer than 100 CFU. This number was significantly higher than those for the control or cortisone-treated group ($P < 0.025$). The difference in the percentage of rats in all three groups with fewer than 100 CFU was significant ($P < 0.05$).

TABLE 2. Macroscopic lung pathology and abscess incidence 2 weeks after intratracheal challenge with *P. aeruginosa* in alginate beads[a]

Treatment group	No. of rats with the following lung pathology score/total no. of rats (%):			Lung abscess incidence (%)
	1 + 2	3	4	
Control	3/20 (15)	6/20 (30)	11/20 (55)	55
Ginseng	10/20 (50)[b,c]	8/20 (40)	2/20 (10)[d,e]	10[d,e]
Cortisone	1/19 (5.3)	4/19 (21)	14/19 (74)	74

[a] A three-by-two chi-square test showed that the differences between the groups in lung abscess incidence ($\chi^2 = 16.97$; $P < 0.001$), a score of 1 + 2 ($\chi^2 = 12.04$, $P < 0.005$), and a score of 4 ($\chi^2 = 16.97$; $P < 0.001$) are significant.
[b] $P < 0.02$ compared to the control group.
[c] $P < 0.01$ compared to the cortisone-treated group.
[d] $P < 0.01$ compared to the control group.
[e] $P < 0.001$ compared to the cortisone-treated group.

Pathology. (i) Macroscopic lung pathology. Abscesses, atelectases, hemorrhages, and fibrinous adhesion to the thoracic wall or diaphragm could be found in all groups of rats 2 weeks after challenge. However, the lung pathology in the ginseng-treated group was significantly milder (Table 2) compared to those in the control and the cortisone-treated groups ($P < 0.02$ and $P < 0.01$, respectively). The differences between the groups for rats with a score of 1 and 2 and those with a score of 4 were significant ($P < 0.005$ and $P < 0.001$, respectively). The incidence of lung abscesses in the ginseng-treated group was also significantly lower than those in the other two groups ($P < 0.01$ and $P < 0.001$, respectively).

(ii) Histopathological changes in the lungs. Significantly milder pathology was found microscopically in the ginseng-treated group (Table 3) compared to those in the cortisone-treated group ($P < 0.04$) and the control group ($P = 0.05$). Acute inflammation in lung tissues was seldom found in the ginseng-treated group. Furthermore, the mast cell number in the ginseng-treated group was significantly lower than those in the other two groups ($P < 0.04$ and $P < 0.004$, respectively) (Table 4), and the ginseng-treated group comprised significantly more rats with a score of i (mast cell number, <150) and fewer rats with a score of iii (mast cell number, >300) compared with those for the control group (sterile saline) ($P < 0.005$). The differences between the groups for rats with a score of i and those with a score of iii were significant ($P < 0.005$ and $P < 0.025$, respectively).

TABLE 3. Microscopic scoring of the lung pathology 2 weeks after challenge with *P. aeruginosa* in alginate beads

Treatment group (no. of animals)	No. (%) of animals with the following:			
	Score 2[a]	Score 3[a]	Score 4[a]	Acute inflammation
Control (10)	5 (50)	2 (20)	3 (30)	3 (30)
Ginseng (10)	9 (90)[b,c]	0 (0)	1 (10)	1 (10)
Cortisone (9)	4 (44)	1 (11)	4 (44)	3 (33)

[a] See Materials and Methods.
[b] $P < 0.04$ compared to cortisone-treated group.
[c] $P = 0.052$ compared with the control group.

TABLE 4. Mast cell count and scoring groups of the inflammatory tissues in rat lungs[a]

Treatment group	No. of rats with the following score/total no. of rats (%)			Median no. of mast cells (range)
	Score i	Score ii	Score iii	
Control	0/10 (0)	3/10 (30)	7/10 (70)	485 (152–1288)
Ginseng	7/10 (70)[b]	2/10 (20)	1/10 (10)[b]	94 (44–429)[b]
Cortisone	2/9 (22)[c]	3/9 (33)	4/9 (44)	268 (96–602)[c]

[a] The total number of mast cells in 10 high-magnification fields (magnification, ×500). A three-by-two chi-square test showed that the differences between the groups with a score of i ($\chi^2 = 11.95$; $P < 0.005$) and a score of iii ($\chi^2 = 7.47$; $P < 0.025$) are significant.
[b] $P < 0.005$ compared to the control group.
[c] $P < 0.05$ compared to the ginseng-treated group.

Serum antibody responses. (i) Antibody responses to *P. aeruginosa* sonicate. Two weeks after challenge, the level of anti-*P. aeruginosa* sonicate IgG antibody in the sera of rats in the ginseng-treated group was significantly lower (Table 5) than that in the control group ($P < 0.01$) and the cortisone-treated group ($P < 0.005$). The IgM level in the cortisone-treated group was increased notably compared to that in the control group ($P < 0.05$) and that in the ginseng-treated group ($P < 0.04$). The IgM level in the ginseng-treated group tended to be lower than that in the control group, but the difference was not significant ($P = 0.06$). No significant difference in serum IgA level was found among the groups.

The IgG2a level in the ginseng-treated group was significantly higher ($P < 0.025$) than that in the control group (Table 6), although a significantly lower total IgG level was found in the ginseng-treated group compared with that in the control group (Table 5). On the other hand, low IgG1 levels were found in both the ginseng- and the cortisone-treated groups, although the difference was not significant compared to the level for the control group.

(ii) Antibody responses to *P. aeruginosa* alginate. No significant difference in levels of antibody to *P. aeruginosa* alginate in serum were found 2 weeks after intratracheal challenge (Table 7).

DISCUSSION

Ginseng is the most well-known and valued herb in China. It has been used as a tonic, for emergency medicine, or as a rejuvenating and revitalizing agent for thousands of years. It is also widely used in other Asian countries, like Japan and the Koreas (11). It has been known that ginseng has a wide range of pharmacological and therapeutic actions on the central nervous, cardiovascular, endocrine, and immune systems (11, 23). However, the potential functions of ginseng as an anti-infectious agent, especially against *P. aeruginosa* pneumonia, and a potential inducer of a TH1-like response have not been reported. Chronic *P. aeruginosa* lung infection with an inflammation dominated by PMNs is a common and troublesome problem for CF patients which has become the leading cause of morbidity and mortality from the disease (7, 27).

The results of our present study showed that ginseng significantly improved the pulmonary bacterial clearance, decreased the severity of lung pathology, reduced lung abscesses, and helped the transformation of pulmonary inflammation from a TH2-like reaction dominated by an abundance of PMNs and a pronounced serum IgG response in the untreated group to a TH1-like response dominated by MNs and a stronger serum IgG2a response in the ginseng-treated group. Ginseng might therefore exhibit promising therapeutic value against *P. aeruginosa* pneumonia. Furthermore, mast cells in the lung foci were found to be noticeably reduced in the ginseng-treated group, and the serum IgG2a level in the ginseng-treated group was significantly higher, although the total IgG level was remarkably lower in the ginseng-treated group than in the control group. These results suggest that ginseng might be an activator of the TH1 response.

The results of protein and endotoxin determinations for the ginseng extract suggest that there are some water-soluble antigens in ginseng. Most herbs and plants including ginseng contain proteins (11), and the endotoxin-like activity might come from the gram-negative rhizobacterial genera associated with roots (24). The cross-reaction of water-soluble antigens in ginseng with *P. aeruginosa* was investigated by crossed immunoelectrophoresis with rabbit hyperimmune antisera raised against water-soluble sonicates of 17 different *P. aeruginosa* O groups (9). By using the same technique, it was investigated whether the rats responded to ginseng treatment by producing antibodies against the compounds present in ginseng after 10 days of ginseng treatment. No cross-reactivity was found between antigens of ginseng and *P. aeruginosa* antigens. No antibodies against ginseng were detected in the treated animals. In a previous work we found that LPS immunization did not help with bacterial clearance, reduce the severity of lung pathology, or change the level of inflammation in the rat model of *P. aeruginosa* lung infection (20). Immune responses can be divided into TH1 and TH2 types. The TH1 type is characterized by the production of antigen-specific IgG2a and the secretion of gamma interferon, interleukin 12 (IL-12), and IL-2, which favor cellular immunity, whereas the TH2 type is asso-

TABLE 5. Serum antibody responses to *P. aeruginosa* sonicate 14 days after challenge with *P. aeruginosa* in rats

Treatment group (no. of animals)	Median ELISA unit (range)		
	IgG	IgA	IgM
Control (20)	1.35 (0.38–4.35)	1.02 (0.33–3.58)	0.31 (0.10–1.00)
Ginseng (20)	0.59 (0.00–2.88)[a]	1.09 (0.30–3.00)	0.17 (0.06–0.77)[b]
Cortisol (19)	1.66 (0.52–4.34)[c]	1.16 (0.62–2.21)	0.60 (0.24–1.18)[d,e]

[a] $P < 0.01$ compared to the control group.
[b] $P < 0.06$ compared to the control group.
[c] $P < 0.005$ compared to the ginseng-treated group.
[d] $P < 0.05$ compared to the control group.
[e] $P < 0.04$ compared to the ginseng-treated group.

TABLE 6. Serum IgG1 and IgG2a responses to *P. aeruginosa* sonicate 14 days after challenge with *P. aeruginosa* PAO 579 in rats

Treatment group (no. of animals)	Median ELISA unit (range)	
	IgG1	IgG2a
Control (20)	0.01 (0–0.73)	0.01 (0–1.30)
Ginseng (20)	0.00 (0–0.22)	0.19 (0–1.89)[a]
Cortisone (19)	0.00 (0–0.29)	0.09 (0–1.45)

[a] $P < 0.025$ compared to the control group.

TABLE 7. Serum antibody responses to the alginate of *P. aeruginosa* 2 weeks after intratracheal challenge with *P. aeruginosa* in rats

Treatment group (no. of animals)	Median ELISA unit (range)		
	IgG	IgA	IgM
Control (20)	0.27 (0–1.35)	0.49 (0.08–1.33)	0.43 (0–1.36)
Ginseng (20)	0.27 (0–1.73)	0.64 (0–1.25)	0.51 (0.08–1.26)
Cortisone (19)	0.53 (0.05–1.36)	0.72 (0.16–2.11)	0.74 (0.06–2.44)

ciated with antigen-specific IgG1 production, the secretion of IL-4 and IL-10, as well as the proliferation of B cells and mast cells, which favor humoral immunity (3, 4, 6, 12, 18, 21, 22, 25). In CF patients, the *P. aeruginosa* lung infection provokes a rapid antibody response; in turn, the immune complexes are formed in the airways, which attract PMNs to the lungs, leading to damage of the lung tissue (8). High incidences of lung abscess and acute inflammation, high IgG levels against *P. aeruginosa* sonicate in serum, more mast cells in the inflammatory foci, and poor bacterial clearance were found in the control and the cortisone-treated groups in our present study, indicating a TH2-like response. In contrast, significantly lower serum IgG levels but higher IgG2a levels against *P. aeruginosa*, lower incidences of lung abscess and acute inflammation, fewer mast cells in lung foci, and much better clearance of bacteria from the lungs were found in the ginseng-treated group, indicating a TH1-like response. The higher number of mast cells and stronger IgG response in the control and the cortisone-treated groups might be related to the secretion of IL-10, a TH2 cytokine which plays a role in stimulating the proliferation and differentiation of mast cells and B cells. Mast cells play a role in the lung defense mechanism by releasing several neutrophil chemoattractants like tumor necrosis factor alpha and initiating neutrophil influx to the lung foci (1). In the present study, it was noticed that more mast cells were followed by more PMNs in the lung foci in the control group, whereas the opposite situation was found in the ginseng-treated group.

The mechanism of the curative effects of ginseng in the rat model of *P. aeruginosa* pneumonia is still unknown. However, from our results we believe that the effect of ginseng in lowering the serum IgG level and enhancing the shift of pulmonary inflammation from PMN to MN infiltration might be one of the reasons. On the other hand, ginseng can significantly activate the phagocytic activities of PMNs (10) and other phagocytes (30), increase natural killer cell activity and the level of lysozyme in serum, and increase the lymphocyte responses to concanavalin A and LPS (30, 31). These properties of ginseng might also be associated with the changes seen in the ginseng-treated group of our study. Cortisone is an anti-inflammatory agent commonly used to treat various diseases, including CF (5, 26). In our study, subcutaneous administration of cortisone showed no effect on *P. aeruginosa* pneumonia. On the other hand, mast cells are involved in the TH2 response, and they were found most frequently along the acute inflammatory foci in our study. Therefore, it might be an indication to apply antihistamine agents in the treatment of chronic *P. aeruginosa* lung infection.

In conclusion, ginseng appears to be a potentially promising remedy for the treatment of chronic *P. aeruginosa* lung infection in CF patients. Further studies to clarify the mechanisms involved in this model are warranted.

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